Claims

[1] A protein containing a modified human DDR2 cytosolic tyrosine kinase domain having an increased autophosphorylation and tyrosine kinase activity, wherein at least one of three tyrosines 736, 740 and 741 in the activation loop of the human DDR2 cytosolic tyrosine kinase domain are modified by inducing phosphorylations of tyrosines, or by independently mutating to phenylalanine, alanine or glycine by a site-directed mutation.

[2] The protein of claim 1, wherein tyrosine 740 in the activation loop of human DDR2 cytosolic tyrosine kinase domain is essentially modified.

[3] The proten of claim 1, wherein tyrosine 740 of the activation loop of the DDR2 cytosolic tyrosine kinase domain is mutated to phenylalanine 740.

[4] A method for preparing a protein containing DDR2 cytosolic tyrosine kinase domain having increased autophosphorylation and tyrosine kinase activity, through phosphorylation of tyrosines at the DDR2 cytosolic tyrosine kinase domain, comprising the following steps of:

- amplifying DNA fragment which encodes an amino acid sequence sufficiently covering a DDR2 cytosolic tyrosine kinase domain protein, and introducing the amplified DNA fragment into a viral expression vector to construct a recombinant viral expression vector for DDR2 cytosolic tyrosine kinase domain protein and generating recombinant virus carrying the DDR2 cytosolic tyrosine kinase domain protein;
- amplifying DNA fragment which encodes an amino acid sequence sufficiently covering a full-length c-Src or c-Src related protein, and introducing the amplified DNA fragment into another separate virus expression vector genome, to construct a recombinant virus expression vector for the c-Src or c-Src related protein and generating recombinant virus carrying the c-Src or c-Src related protein;
- infecting the obtained virus carrying the DDR2 cytosolic tyrosine kinase domain and the obtained virus carrying the c-Src or c-Src related protein into a host cell, co-expressing the proteins together, and inducing a tyrosine phosphorylation at the DDR2 cytosolic tyrosine kinase domain by the tyrosine kinase activity of c-Src or c-Src related protein, to produce a large amount of a protein containing the DDR2 cytosolic tyrosine kinase domain with increased tyrosine phosphorylation;

- isolating and purifying the obtained protein containing the DDR2 cytosolic tyrosine kinase domain with increased tyrosine phosphorylation.

[5] The method of claim 4, the c-Src related protein is selected from the group consisting of v-Src ,Fyn, Yes, Lck, Hck, Lyn, Csk and Blk including their tyrosine kinase-activated versions.

[6] The method of claim 4, wherein the DDR2 cytosolic tyrosine kinase domain protein is tagged with one selected from the group consisting of glutathione-S-tranferase, thioredoxin or histidine oligomer.

[7] The method of claim 4, wherein the virus carrying the DDR2 cytosolic tyrosine kinase domain protein and the virus carrying the c-Src or c-Src related protein are are simultaneously infected into the host cell at the combination retio of 1:3 to 3:1 and the MOI (multiplicity of infection) of 1 to 10.

[8] The method of claim 4, wherein the virus is a baculovirus and the host cell is an insect cell.

[9] The method of claim 4, wherein the DDR2 cytosolic tyrosine kinase domain is human DDR2 cytosolic tyrosine kinase domain, and at least one of three tyrosines 736, 740 and 741 of human DDR2 cytosolic tyrosine kinase domain are selectively phosphorylated.

[10] The method of claim 9, wherein tyrosine 740 of human DDR2 cytosolic tyrosine kinase domain is essentially phosphorylated.

[11] A method for preparing a protein containing a DDR2 cytosolic tyrosine kinase domain having an increased autophosphorylation and tyrosine kinase activity, by phosphorylating tyrosine at the DDR2 cytosolic tyrosine kinase domain protein, comprising the following steps of:

- amplifying DNA fragment which encodes an amino acid sequence sufficiently covering a DDR2 cytosolic tyrosine kinase domain protein, and introducing the amplified DNA fragment into a viral expression vector to construct a recombinant viral expression vector for DDR2 cytosolic tyrosine kinase domain protein and generating recombinant virus carrying the DDR2 cytosolic tyrosine kinase domain protein;

- Infecting the obtained the virus of the DDR2 cytosolic tyrosine kinase domain into a host cell, to produce a protein containing the DDR2 cytosolic tyrosine kinase domain, and then treating the cells with H_2O_2 at the concentration of 10 μM to 1 mM to induce tyrosine phosphorylation at

the expressed DDR2 cytosolic tyrosine kinase domain; and
- isolating and purifying the expressed protein containing the DDR2 cytosolic tyrosine kinase domain with induced tyrosine phosphorylation.

[12] The method of claim 11, wherein the DDR2 cytosolic tyrosine kinase domain protein is tagged with one selected from the group consisting of glutathione-S-tranferase, thioredoxin or histidine oligomer.

[13] The method of claim 11, wherein the virus is a baculovirus and thehost cell is an insect cell.

[14] The method of claim 11, wherein the DDR2 cytosolic tyrosine kinase domain is human DDR2 cytosolic tyrosine kinase domain, and at least one of three tyrosines 736, 740 and 741 of human DDR2 cytosolic tyrosine kinase domain are selectively phosphorylated.

[15] The method of claim 14, wherein tyrosine 740 of human DDR2 cytosolic tyrosine kinase domain is essentially phosphorylated.

[16] A method for preparing a protein containing a DDR2 cytosolic tyrosine kinase domain having an increased autophosphorylation and tyrosine kinase activity, by mutating at least one of tyrosines at the DDR2 cytosolic tyrosine kinase domain, comprising the following steps of:
- amplifying DNA fragment which encodes an amino acid sequence sufficiently covering a DDR2 cytosolic tyrosine kinase domain protein where at least one of tyrosines at the DDR2 cytosolic tyrosine kinase domain are independently mutated to phenylalanine, alanine or glycine, by a site-directed mutagenesis, and introducing the amplified DNA fragment into a viral expression vector to construct a recombinant viral expression vector for DDR2 cytosolic tyrosine kinase domain with mutation of at least one tyrosine to phenylalanine, alanine or glycine, and generating recombinant virus carrying the mutant DDR2 cytosolic tyrosine kinase domain ;
- infecting the obtained reconbinant virus of the mutant DDR2 cytosolic tyrosine kinase domain into a host cell, to produce a protein containing the mutant DDR2 cytosolic tyrosine kinase domain,
- isolating and purifying the expressed mutant protein containing the DDR2 cytosolic tyrosine kinase domain with mutation of at least one of tyrosines to phenylalanine, alanine or glycine.

[17] The method of claim 16, wherein the DDR2 cytosolic tyrosine kinase domain protein is tagged with one selected from the group consisting of

glutathione-S-tranferase, thioredoxin or histidine oligomer.

[18] The method of claim 16, wherein the virus is a baculovirus and thehost cell is an insect cell.

[19] The method of claim 16, wherein the DDR2 cytosolic tyrosine kinase domain is human DDR2 cytosolic tyrosine kinase domain, and at least one of three tyrosines 736, 740 and 741 of human DDR2 cytosolic tyrosine kinase domain are independently mutated to phenylalanine, alanine or glycine.

[20] The method of claim 14, wherein tyrosine 740 of the DDR2 cytosolic tyrosine kinase domain is essentially mutated to phenylalanine, alanine or glycine.

[21] A use of a protein containing a modified DDR 2 cytosolic tyrosine kinase domain having an increased autophosphorylation and tyrosine kinase activity, to be utilized in developing medical drugs for treating a disease caused by an excessive autophoshorylation and tyrosine kinase activity of DDR2 protein, wherein at least one tyrosine of the activation loop of human DDR2 cytosolic tyrosine kinase domain are modified by inducing phosphorylations of tyrosines, or by independently mutating to phenylalanine, alanine or glycine by a site-directed mutation.

[22] The use of claim 21, wherein the DDR2 cytosolic tyrosine kinase domain is human DDR2 cytosolic tyrosine kinase domain, and at least one of three tyrosines 736, 740 and 741 of human DDR2 cytosolic tyrosine kinase domain are modified.